Ecotoxicology and Environmental Safety 84 (2012) 99–103

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Baseline values of Micronuclei and Comet Assay in the lizard *Tupinambis merianae* (Teiidae, Squamata)

Laura G. Schaumburg [a,b,e,*,1], Gisela L. Poletta [a,b,c,e,1,2], Pablo A. Siroski [b,d,e], Marta D. Mudry [a,e,1]

[a] Grupo de Investigación en Biología Evolutiva (GIBE), IEGEBA (CONICET-UBA), Labs. 43-46, Piso 4, FCEyN, Pabellón II, Cdad. Universitaria, Intendente Güiraldes 2160, CP: 1428EGA, Buenos Aires, Argentina

[b] Laboratorio de Zoología Aplicada: Anexo Vertebrados, Facultad de Humanidades y Ciencias, UNL/Ministerio de Aguas, Servicios Públicos y Medio Ambiente, Aristóbulo del Valle 8700, CP: 3000, Santa Fe, Argentina

[c] Cátedra de Toxicología, Farmacología y Bioquímica Legal, Facultad de Bioquímica y Ciencias Biológicas, UNL, CC 242 Paraje El Pozo, CP: 3000, Santa Fe, Argentina

[d] Dir. Gral. Manejo Sustentable Fauna y Flora, Aristóbulo del Valle 8700, CP: 3000, Santa Fe, Argentina

[e] National Council of Scientific and Technical Research (CONICET), Argentina

ARTICLE INFO

Article history:
Received 25 January 2012
Received in revised form
20 May 2012
Accepted 25 June 2012
Available online 14 August 2012

Keywords:
Genotoxicity
Reptiles
Sentinel species
Tegu lizard

ABSTRACT

The Micronucleus test (MN) and Comet assay (CA) are currently the most widely used methods that allow the characterization of DNA damage induced by physical and chemical agents in wild species. The continuous expansion of the cultivated areas in Argentina, since the introduction of transgenic crops, mainly soy, in association with the increased use of pesticides, transformed deeply the natural environments where the lizard *Tupinambis merianae* (tegu lizard) occurs. Despite the fact that reptiles have shown to be excellent bioindicators of environmental contaminants, there is no record of genotoxicity studies in *T. merianae*. The aim of the present study was to adjust the MN test and CA protocols to be applied in erythrocytes of *T. merianae*, and determine the baseline values of DNA damage in this species. We used 20 adult lizards (10 males: 10 females) from Estación Zoológica Experimental "Granja La Esmeralda" (Santa Fe, Argentina). Peripheral blood samples were collected from all animals and the MN test and CA applied according to the protocols established for other reptilian species. We test critical parameters of CA protocol (cell density, unwinding and electrophoresis times) using increasing concentrations of H_2O_2 (10, 25 and 50 μM) as a known genotoxic agent to induce DNA damage. Based on this, we determined the most suitable conditions for the CA in this species: a cell density of 4×10^3 erythrocytes per slide, 10 min of *unwinding* and 15 min of electrophoresis at 0.90 V/cm approximately. The baseline frequency of micronuclei (BFMN = MN/1000 erythrocytes counted) determined for this species was 0.95 ± 0.27 and the basal damage index (BDI: calculated from 100 comet images classified in arbitrary units) = 103.85 ± 0.97. No differences were observed between sexes in the BFMN or BDI ($p > 0.05$), and no relation was found between baseline values and length or weight of the analyzed animals ($p > 0.05$).

These results demonstrated the sensitivity of both biomarkers of genotoxicity to be applied in erythrocytes of this species, with baseline values comparable to those reported in other reptilian species. These results allow us to propose the tegu lizard for future in vivo studies to assess the genotoxicity of different agents, including those possibly affecting it in its natural geographic distribution.

1. Introduction

Tupinambis merianae (tegu lizard) is endemic to South America (Presch, 1973) and is one of the two native species of the genus *Tupinambis* (Teiidae, Squamata) inhabiting Argentina (Ávila-Pire, 1995). This lizard is widely distributed in the eastern and north-central region of the country (Fig. 1) and has an annual reproductive season that takes place from October to December. Females build their nests in caves dug into the soil, carefully prepared and isolated from major climate changes (Yanosky and Mercolli, 1992).

Populations of the genus *Tupinambis* living in Argentina are included in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). Since 2007, *T. merianae* has been under management on a sustainable use program in Santa Fe province (Argentina), known as "Proyecto Iguana".

* Corresponding author at: Laboratorio de Zoología Aplicada: Anexo Vertebrados, Facultad de Humanidades y Ciencias, UNL/Ministerio de Aguas, Servicios Públicos y Medio Ambiente, Aristóbulo del Valle 8700, CP: 3000, Santa Fe, Argentina.
E-mail address: giseschaumburg@yahoo.com.ar (L.G. Schaumburg).
[1] Fax: 5411 4576 3354.
[2] Fax: 54 342 4579256.

0147-6513/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ecoenv.2012.06.023

Fig. 1. Geographic distribution of *T. merianae* in Argentina.

This program is based on the ranching technique, which implies the collection of eggs from natural environment, subsequent artificial incubation, birth and raising of the animals under controlled conditions until they reach an appropriate size to be released into the wild and to avoid predation or influence by very low temperatures. Twenty-five per cent of the animals born are used for commercial purpose as pets.

The natural environment where the tegu lizard occurs in Argentina has been highly transformed as a result of the fast and continuous expansion of the cultivated areas into new regions, since the introduction of transgenic crops, mainly soy, and the implementation of non-tillage system (Paruelo et al., 2006; Aizen et al., 2009). This has lead to deforestation, drainage of wetlands to obtain more lands for agriculture, and contamination of natural environments through increasing pesticides application.

Among the short-term test applied as biomarkers of genotoxicity in wild species, the Micronucleus test (MN) and Comet assay (CA) are preferred methods due to their sensitivity to detect chromosomal and DNA damage induced by physical and chemical agents at an early stage, the possibility to apply them in any nucleated cell type, and the small sample required (Mitchelmore and Chipman, 1998; Carballo and Mudry, 2006). The sensitivity of these tests may be compared although both methods measure different endpoints and, as a result, they complement each other (Cotelle and Férard, 1999; Ohe et al., 2004).

Although reptiles have shown to be excellent bioindicators of contamination (Mitchelmore et al., 2005), they remain as the group of vertebrates less studied in genetic toxicology. In the literature, few studies have successfully applied the MN test and CA in reptile erythrocytes to determine basal values of DNA damage (Zúñiga-González et al., 2001; Boned et al., 2011), and for in vivo evaluation of genotoxicity induced by physical (Schaumburg et al., 2010) or chemical agents (Poletta et al., 2009; Capriglione et al., 2011). To our knowledge, there is no report of the application of these biomarkers in *T. merianae*.

The aim of the present study was to optimize two widely used genotoxic biomarkers, the MN test and CA, to be applied in erythrocytes of *T. merianae*, in order to determine the baseline values of DNA damage and to propose the tegu lizard as a sentinel species to characterize genotoxic effects in natural environments.

2. Materials and methods

2.1. Chemicals

Dimethyl sulfoxide (DMSO) was purchased from Fluka. Low melting point agarose (LMP), normal melting point agarose (NMP), ethidium bromide (EB), acridine orange (AO), Hydrogen Peroxide (H_2O_2), and the rest of the reagents for the Comet and MN assays were provided by Sigma. RPMI-1640 medium was from HyClone and Giemsa was from Biopur®.

2.2. Animals

Twenty *T. merianae* adults, 10 males and 10 females (37.50–51.50 cm) from Estación Zoológica Experimental "Granja La Esmeralda" (Santa Fe, Argentina), were used for this study.

All lizards were weighted (Dixie Kretz, precision 1 g) and measured in snout-vent length (SVL, precision 0.1 cm). The sex of the animals was externally determined by the presence of scales on the cloacal region and hypertrophied muscles in the neck region forming a conspicuous chin in adult males (Donadío and Gallardo, 1984).

Peripheral blood samples (1 ml) were obtained from the caudal vein of all animals, as described by Olson et al. (1977), with heparinized syringes and 25G × 1″ needles.

2.3. Micronucleus test (MN)

The MN test was performed according to Poletta et al. (2008), with some modifications as follows: two blood smears were made for each animal on clean glass slides, fixed with methanol for 10 min, and then stained with Giemsa 10% for 10 min. Giemsa solution was centrifuged and filtered before staining to reduce precipitations that could interfere with the analysis. Slides were coded for 'blind' analysis and then examined under the microscope Olympus CX21 at 1000X. For the identification of MN we used the criteria described by Schmid (1975): (i) micronuclei size should be between one-tenth and one-third of the diameter of the main nucleus, (ii) they should be on the same plane of focus, (iii) they should have the same color, texture and refraction as the main nucleus and (iv) they should be clearly separated from the main nucleus.

For each animal, 1000 cells were analyzed and the basal frequency of MN (BFMN=number of cells with MN/1000 cells counted) was recorded.

2.4. Comet assay (CA)

Cell viability was determined before the application of CA by fluorescent DNA-binding dyes. A cell suspension was mixed with a dye-mix working solution of 100 μg/ml AO and 100 μg/ml EB, prepared in Ca^{2+}- and Mg^{2+}-free PBS (phosphate buffered saline), and then examined under the fluorescent microscope Leica DMLB at 400X. A total of 100 cells were counted per sample and the percentage of viable cells was determined (Mercille and Massie, 1994).

Since there was no previous report of the application of the CA in *T. merianae*, we had to carry out a preliminary study using the protocol previously established for erythrocytes of another reptile species (Poletta et al., 2008), testing critical parameters such as cell suspension, unwinding and electrophoresis conditions. We used increasing concentrations of H_2O_2 as a known genotoxic agent (10, 25 and 50 μM) to induce strand breaks in vitro during 30 min, and distilled water as a negative control. Blood samples were diluted 1:19 (v/v) with RPMI 1640 and 1.5 μL of the cell suspension (4.0×10^3 erythrocytes, approximately) was used to prepare each of two slides per blood sample, following the referred protocol. Slides were immersed in lysis buffer for 24–48 h. Unwinding and electrophoresis times tested were (a) 5 min each, (b) 20 min each, and (c) 10 min unwinding, 15 min electrophoresis. Electrophoresis conditions were always 300 mA and 25 V (0.90 V/cm) (Poletta et al., 2008).

Once we have set the proper conditions for the application of the CA in erythrocytes of the tegu lizard, we applied it in untreated blood samples to determine the basal damage index.

All slides were coded for blind analysis, they were stained with EB (2 μg/ml), and 100 randomly selected comet images (50 from each replicate) were analyzed per animal under the fluorescent microscope. The comets were visually classified into five classes according to tail size and intensity (from undamaged, class 0, to maximally

damaged, class 4), resulting in a baseline damage index (BDI = $1n_1+2n_2+3n_3+4n_4$) per animal (Rodríguez Ferreiro et al., 2002; Poletta et al., 2008).

2.5. Statistical analysis

The statistical analysis was conducted with the software SPSS 14.0 for Windows. We used the ANOVA followed by Tukey test for the comparison of H_2O_2 treated groups with the negative control. Mean ± Standard deviation (SD) of BFMN and BDI were calculated from data of all animals. We used the Student *t*-test for the comparison of BFMN and BDI between males and females. Linear regressions were carried out to analyze the relation between BFMN and BDI with size of the animals. A $p < 0.05$ was considered statistically significant.

3. Results

In the MN test, the protocol and staining used for blood smears resulted in a clear visualization of the MN in the erythrocytes of the species, avoiding confusion (Fig. 2).

Regarding CA, cell viability of all samples was in the range of 95–100%, indicating appropriate conditions for the application of this technique in tegu lizard. After the comparison of the extent of DNA migration in control and treated cells at 3 different unwinding/electrophoresis times tested, we detected that the best time duration for unwinding and electrophoresis was 10 and 15 min respectively, as we obtained the characteristic comet shape in the case of the positive controls and a minimum migration in the case of negative controls (Fig. 3). Five minutes was not enough for unwinding/electrophoresis steps, giving a minimum of DNA migration even in the cells treated with the highest concentration of H_2O_2.On the other hand, conducting both steps during 20 min resulted in an excessive migration, with comet tails separated from the nucleoids in most of the damaged cells. A significant increase in DNA damage was observed at all concentrations of H_2O_2 assayed: 10 μM = 178 ± 24.75; 25 μM = 173 ± 17.32; 50 μM = 187.67 ± 32.35, in relation to the negative control: 119 ± 24.04 ($p < 0.05$), but without a dose–response relationship.

Total values and mean (± SD) of BFMN and BDI for all animals are presented in Table 1. The baseline values determined for this species were BFMN = 0.95 ± 0.27 and BDI = 103.85 ± 0.97. No differences were observed between males and females in the BFMN or BDI ($p > 0.05$), and no relation was found between baselines values and length or weight of the animals ($p > 0.05$).

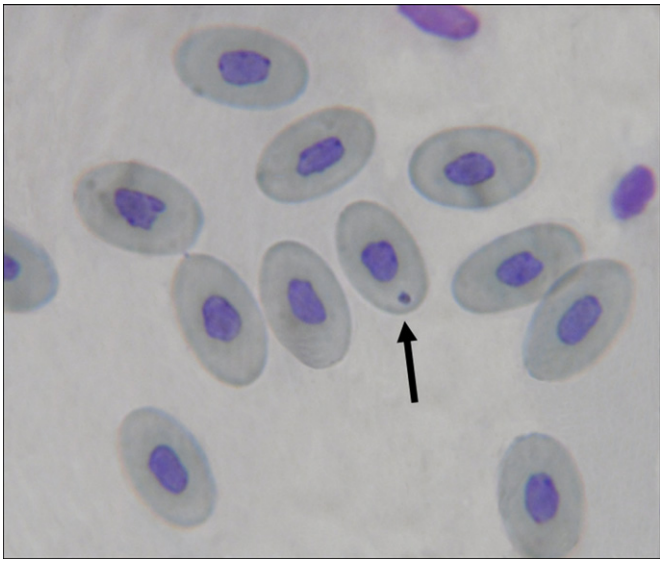

Fig. 2. *T. merianae* micronucleated erythrocytes (arrow) stained with Giemsa (1000X).

Table 1
Basal frequency of MN and baseline damage index values for *T. merianae*.

Animal	Sex	BFMN[a]	BDI[b]
1	♂	5	116
2	♀	0	102
3	♂	1	110
4	♂	1	102
5	♂	2	111
6	♂	1	100
7	♂	0	101
8	♀	2	108
9	♂	2	102
10	♂	1	104
11	♂	0	100
12	♀	0	104
13	♂	0	105
14	♀	1	100
15	♀	0	102
16	♀	1	104
17	♀	1	100
18	♀	1	102
19	♀	0	100
20	♀	0	104
X ± SD		0.95 ± 0.27	103.85 ± 0.97

[a] Basal frequency of micronucleus (MN/1000 cells counted).
[b] Baseline damage index (determined by the analysis of 100 comet images classified in arbitrary units).

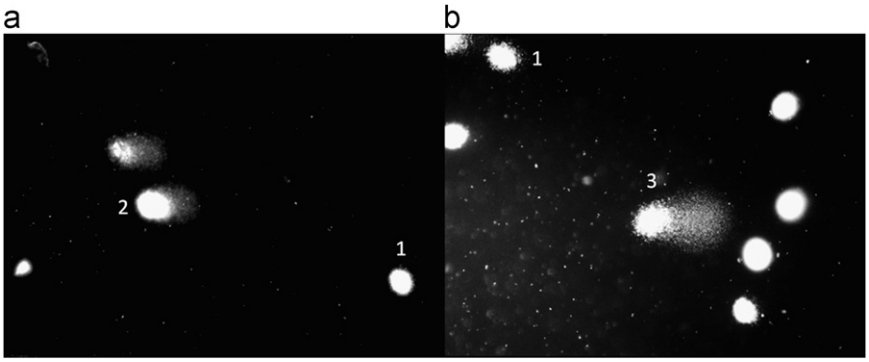

Fig. 3. *T. merianae* comet images of different DNA damage classes obtained from peripheral blood erythrocytes: (a) negative control: class 1 and 2, (b) H_2O_2 10 μM: class 1 and 3 (400X).

4. Discussion

The MN test and CA are widely used in biomonitoring studies to detect DNA damage in different wild species such as fish (Vera Candioti et al., 2010; Ansari et al., 2011), amphibians (Lajmanovich et al., 2005; Hui Yin et al., 2008), reptiles (Poletta et al., 2009; Strunjak-Perovic et al., 2010; Poletta et al., 2011) and mammals (Heuser et al., 2002). In all cases, they demonstrated a high sensitivity to detect the effects of genotoxic agents.

The present study explored the utility of the MN test and CA for genotoxic evaluation in *T. merianae*, being the first report on their application in erythrocytes of this reptilian species. We set the suitable cell density, unwinding and electrophoresis conditions to apply the CA technique in this species, which is both, species- and cell type-specific (Lee and Steinert, 2003). Besides, we determined baseline values of MN and DI for this reptilian species. This information would be very useful as reference values for future in vivo studies to assess the genotoxicity of different agents in the tegu lizard.

According to Grisolia et al. (2009) the differences observed in the baseline DNA damage among the species living in the same environment show that we must be aware of the differential sensitivity of organisms. Udroiu (2006) indicated that intrinsic factors like lifespan of circulating erythrocytes, and removal time of old or damaged erythrocytes could influence the presence of spontaneous MN. On the other hand, the sensitivity of genotoxicity tests may also be affected by high inter-individual variability (Akcha et al., 2003). Ectothermic organisms such as lizards, which have a low metabolic rate, can be more sensitive to the effects of xenobiotics and their recovery may be slower than other non-reptilian species (Hall, 1980). In this context, the basal level of DNA damage has been shown to be influenced by multiple factors and the literature suggests that it must be evaluated according to the species, sex, and age (Zúñiga-González et al., 2000, 2001). In the present study, no differences were found between animals of different ages and sex in the BFMN or BDI, suggesting that basal values are stable among animals in this species.

Zúñiga-González et al. (2001) reported baseline MN level for two species of lizards, *Iguana iguana* and *Ctenosaura pectinata* (0.10 and 0.05/1000, respectively), and for other reptilian species such as snakes, turtles and crocodiles (BFMN from 0 to 0.30/1000) (Zúñiga-González et al., 2000, 2001). Similar results were obtained for the snake *Hierophis gemonensis* (0.30/1000) (Strunjak-Perovic et al., 2010). These values were considerably lower than the BFMN found in our study for *T. merianae* (0.95 ± 0.27). Large differences in MN frequencies between studies can result from different analyzers and the low number of individuals used in the cited works.

Our results agree with those reported for the broad-snouted caiman (*Caiman latirostris*) both in BFMN (0.87 ± 0.74) and BDI (103.40 ± 3.36) (Poletta et al., 2008). Furthermore, we found no relation between BFMN or BDI with sex or size of *T. merianae*, as it was reported by Poletta et al. (2008) for *C. latirostris*.

During the last years, several wild species have been used as bioindicators in the monitoring of environmental quality (Heuser et al., 2002; Wirzinger et al., 2007; Bosch et al., 2011), taking into account that their susceptibility to different xenobiotics can be used as early warnings of environmental alterations (Burlibaşa and Gavrilă, 2011).

The results obtained in the present contribution have demonstrated that the tegu lizard could be a good indicator for in vivo genotoxicity assessment of contaminants through the CA and MN test. Besides, the possibility to take blood samples without causing any damage to these animals implies a great advantage for future studies in a wild species under management, as is *T. merianae*. Further studies are necessary to determine this species as a sentinel organism for the evaluation of those compounds affecting it in its natural geographic distribution.

5. Conclusions

The information provided in the present study confirms the sensitivity of the MN test and CA to be applied as biomarkers of genotoxicity in erythrocytes of *T. merianae*.

Considering that *T. merianae* is an endemic species to South America together with the existence of a sustainable use program on it in Argentina, and the demonstrated sensitivity for the application of MN test and CA in this species, we propose the tegu lizard for future in vivo studies to assess the genotoxicity of different agents, including those possibly affecting this species in its natural geographic distribution.

Acknowledgments

This study was supported by Laboratorio de Zoología Aplicada: Anexo Vertebrados (Facultad de Humanidades y Ciencias, UNL/MASPyMA), Universidad de Buenos Aires (MDM PIP 112 CONICET, Buenos Aires, Argentina) and Proyecto Iguana (Res. 031/07, Secretaria de Estado de Medio Ambiente y Desarrollo Sustentable de la Provincia de Santa Fe). We would like to thank members of Proyecto Iguana and Proyecto Yacaré for their help in this study. This study is part of LGS research as a doctoral fellow of CONICET in work collaboration between the UNL/MASPyMA and UBA.

References

Aizen, M., Garibaldi, L.A., Dondo, M., 2009. Expansión de la soja y diversidad de la agricultura argentina. Ecol. Austral 19, 45–54.

Akcha, F., Vincent Hubert, F., Pfhol-Leszkowicz, A., 2003. Potential value of the comet assay and DNA adduct measurement in dab (*Limanda limanda*) for assessment of in situ exposure to genotoxic compounds. Mutat. Res. 534, 21–32.

Ansari, R.A., Rahman, S., Kaur, M., Anjum, S., Raisuddin, S., 2011. In vivo cytogenetic and oxidative stress-inducing effects of cypermethrin in freshwater fish, *Channa punctata* Bloch. Ecotoxicol. Environ. Saf. 74, 150–156.

Ávila-Pire, T.C.S., 1995. Lizards of Brazilian Amazonia (Reptilia: Squamata). Zool. Verhandelinger 299, 1–706.

Boned, M.J., López González, E.C., Latorre, M.A., Poletta, G.L., Siroski, P.A., 2011. Determinación del valor basal de micronúcleos (MN) en la tortuga de laguna (*Phrynops hilarii*). J. Basic Appl. Genet. 40, 91.

Bosch, B., Mañas, F., Gorla, N., Aiassa, D., 2011. Micronucleus test in post metamorphic *Odontophrynus cordobae* and *Rhinella arenarum* (Amphibia: Anura) for environmental monitoring. J. Toxicol. Environ. Health Sci. 3, 155–163.

Burlibaşa, L., Gavrilă, L., 2011. Amphibians as model organisms for study environmental genotoxicity. Appl. Ecol. Environ. Res. 9, 1–15.

Capriglione, T., De Iorio, S., Gay, F., Capaldo, A., Vaccaro, M.C., Morescalchi, M.A., Laforgia, V., 2011. Genotoxic effects of the fungicide thiophanate-methyl on *Podarcis sicula* assessed by micronucleus test, comet assay and chromosome analysis. Ecotoxicology 20, 885–891.

Carballo, M.A., Mudry, M.D., 2006. Indicadores y Marcadores Biológicos. In: Mudry, M.D., Carballo, M.A. (Eds.), Genética Toxicológica, Editorial De los Cuatro Vientos. Buenos Aires, pp. 83–107.

Cotelle, S., Férard, J.F., 1999. Comet assay in genetic ecotoxicology: a review. Environ. Mol. Mutagenesis 34, 246–255.

Donadío, O., Gallardo, J.M., 1984. Biología y conservación de las especies del género Tupinambis (Squamata, Sauria, Teiidae) en Argentina. Rev. Mus. Argent. de Cienc. Nat. Bernadino Rivadavia1 13, 117–127.

Grisolia, C.K., Rivero, C.L.G., Starling, F.L.R.M., da Silva, I.C.R., Barbosa, A.C., Dorea, J.G., 2009. Profile of micronucleus frequencies and DNA damage in different species of fish in a eutrophic tropical lake, Gen. Mol. Biol. 32, 138–143.

Hall, R.J., 1980. Effects of Environmental Contaminants on Reptiles: A review. Special Scientific Report—Wildlife no. 228. U.S. Department of the Interior Fish and Wildlife Service, Washington D.C., pp. 1–12.

Heuser, V.D., da Silva, J., Moriske, H-J., Dias, J.F., Yoneama, M.L., de Freitas, T.R.O., 2002. Genotoxicity biomonitoring in regions exposed to vehicle emissions using the comet assay and the micronucleus tests in native rodent *Ctenomys minutes*. Environ. Mol. Mutagenesis 40, 227–235.

Hui Yin, X., Li, S.N., Zhang, L., Zhu, G.N., Zhuang, H.S., 2008. Evaluation of DNA damage in Chinese toad (*Bufo bufo gargarizans*) after in vivo exposure to

sublethal concentrations of four herbicides using the comet assay. Ecotoxicology 17, 280–286.
Lajmanovich, R.C., Cabagna, M., Peltzer, P.M., Stringhini, G.A., Attademo, A.M., 2005. Micronucleus induction in erythrocytes of the *Hyla pulchella* tadpoles (Amphibia: Hylidae) exposed to insecticide Endosulfan. Mutat. Res. 587, 67–72.
Lee, R.F., Steinert, S., 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutat. Res. 544, 43–64.
Mercille, S., Massie, B., 1994. Induction of apoptosis in nutrient-deprived cultures of hibridoma and myeloma cells. Biotechnol. Bioeng. 44, 1140–1154.
Mitchelmore, C.L., Chipman, J.K., 1998. DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. Mutat. Res. 399, 135–147.
Mitchelmore, C.L., Rower, C.L., Place, A.R., 2005. Tools for assessing contaminant exposure and effects in reptiles. In: Gardner, S.C., Oberdörster, E. (Eds.), Toxicology of Reptiles. New perspective: Toxicology and the Environment. Taylor & Francis Group, Florida, pp. 63–122.
Ohe, T., Watanabe, T., Wakabayashi, K., 2004. Mutagens in surface waters: a review. Mutat. Res. 567, 109–149.
Olson, G., Hessler, J., Faith, R., 1977. Techniques for the blood collection and intravascular infusion of reptiles. Lab. Anim. Sci. 25, 783–786.
Paruelo, J.M., Guerschman, J.P., Piñeiro, G., Jobbágy, E.G., Verón, S.R., Baldi, G., Baeza, S., 2006. Cambios en el uso de la tierra en Argentina y Uruguay: marcos conceptuales para su análisis. Agrociencia 10, 47–61.
Poletta, G.L., Larriera, A., Kleinsorge, E., Mudry, M.D., 2008. *Caiman latirostris* (broad-snouted caiman) as a sentinel organism for genotoxic monitoring: basal values determination of micronucleus and comet assay. Mutat. Res. 650, 202–209.
Poletta, G.L., Larriera, A., Kleinsorge, E., Mudry, M.D., 2009. Genotoxicity of the herbicide formulation Roundup® (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test. Mutat. Res. 672, 95–102.
Poletta, GL., Kleinsorge, E., Paonessa, A., Mudry, M.D., Larriera, A., Siroski, P.A., 2011. Genetic, enzymatic and developmental alterations observed in *Caiman latirostris* exposed *in ovo* to pesticide formulations and mixtures in an experiment simulating environmental exposure. Ecotoxicol. Environ. Saf. 74, 852–859.
Presch, W., 1973. A review of the tegu lizards genus Tupinambis (Sauria: Teiidae) from South America. Copeia 4, 740–746.
Rodríguez Ferreiro, G., Cancino Badías, L., López-Nigro, M., Palermo, A., Mudry, M.D., Prieto González, E., Carballo, M., 2002. DNA single strand breaks in peripheral blood lymphocytes induced by three nitroimidazole derivatives. Toxicol. Lett. 132, 109–115.
Schaumburg, L.G., Poletta, G.L., Imhof, A., Siroski, P.A., 2010. Ultraviolet radiation-induced genotoxic effects in the broad-snouted caiman, *Caiman latirostris*. Mutat. Res. 700, 67–70.
Schmid, W., 1975. The micronucleus test. Mutat. Res. 31, 9–15.
Strunjak-Perovic, I., Lisicic, D., Coz-Rakovac, R., Topic Popovic, N., Jada, M., Benkovi, V., Tadic, Z., 2010. Evaluation of micronucleus and erythrocytic nuclear abnormalities in Balkan whip snake *Hierophis gemonensis*. Ecotoxicology 19, 1460–1465.
Udroiu, I., 2006. The micronucleus test in piscine erythrocytes. Aquat. Toxicol. 79, 201–204.
Vera Candioti, J., Soloneski, S., Larramendy, M.L., 2010. Genotoxic and cytotoxic effects of the formulated insecticide Aficida® on *Cnesterodon decemmaculatus* (Jenyns, 1842) (Pisces: Poeciliidae). Mutat. Res. 703, 180–186.
Wirzinger, G., Weltje, L., Gercken, J., Sordyl, H., 2007. Genotoxic damage in field-collected three-spined sticklebacks (*Gasterosteus aculeatus* L.): a suitable biomonitoring tool? Mutat. Res. 628, 19–30.
Yanosky, A., Mercolli, C., 1992. Tegu lizard (*Tupinambis teguixin*) management in captivity at El Bagual Ecological Reserve, Argentina. Arch. Zootec. 41, 41–51.
Zúñiga-González, G., Torres-Bugarín, O., Luna-Aguirre, J., González-Rodríguez, A., Zamora-Perez, A., Gómez-Meda, B.C., Ventura-Aguilar, A.J., Ramos-Ibarra, M.L., Ramos-Mora, A., Ortíz, G.G., Gallegos-Arreola, M.P., 2000. Spontaneous micronuclei in peripheral blood erythrocytes from 54 animal species (mammals, reptiles and birds): part two. Mutat. Res. 467, 99–103.
Zúñiga-González, G., Torres-Bugarín, O., Zamora-Perez, A., Gómez-Meda, B.C., Ramos Ibarra, M.L., Martínez-González, S., González-Rodríguez, A., Luna-Aguirre, J., Ramos-Mora, A., Ontiveros-Lira, D., Gallegos-Arreola, M.P., 2001. Differences in the number of micronucleated erythrocytes among young and adult animals including humans. Spontaneous micronuclei in 43 species. Mutat. Res. 494, 161–167.